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# Effect of the thyroid on faecal shedding of *E. coli* O157:H7 and *Escherichia coli* in naturally infected yearling beef cattle*

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## ABSTRACT

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**Aims:** To determine if thyroid function affects faecal shedding of *Escherichia coli* O157:H7.

**Methods and Results:** Eight yearling cattle ($n = 4$ per treatment group), previously identified as shedding *E. coli* O157:H7, received either 0 or 10 mg 6-*N*-propyl-2-thiouracil (PTU) $kg^{-1}$ BW $day^{-1}$ for 14 days to reduce serum concentrations of the thyroid hormones, $T_3$ and $T_4$. Animals were monitored daily for changes in faecal shedding of *E. coli* O157:H7 and *E. coli* (EC) for the 14-day treatment period and an additional 7 days post-treatment. Body weight was measured weekly and serum concentrations of $T_3$ and $T_4$ were determined every 3 days. No differences in faecal shedding of *E. coli* O157:H7 were observed during the 14-day treatment period. However, compared with control animals, a greater percentage of PTU-treated cattle ejected *E. coli* O157:H7 on day 16 (100 *vs* 25%) and 18 (75 *vs* 0%) of the post-treatment period. Serum $T_3$ was lower in PTU-treated cattle during the 14-day treatment period and greater on day 18 of the post-treatment period.

**Conclusion:** Cattle with chemically altered thyroid hormones had similar shedding patterns of faecal *E. coli* O157:H7 and EC during the 14-day treatment period. However, faecal shedding of *E. coli* O157:H7 tended to be greater, and serum concentrations of $T_3$, were greater for PTU-treated cattle immediately following the termination of PTU treatment.

**Significance and Impact of the Study:** Short-term chemical inhibition of thyroid hormones had minimal effects on faecal shedding of *E. coli* O157:H7 in naturally infected cattle. However, a hyperthyroid state as observed postdosing might play a role in the seasonal shedding of *E. coli* O157:H7 in cattle.

**Keywords:** beef cattle, *Escherichia coli* O157:H7, thyroid hormones.

## INTRODUCTION

The majority of outbreaks of *Escherichia coli* O157:H7 in humans has resulted from foods that originate from cattle, usually ground beef (Gansheroff and O'Brien 2000). A greater incidence of illness occurs during the warmer months (Rangel *et al.*, 2005), which may be correlated with seasonal changes in shedding patterns of *E. coli* O157:H7 observed in various livestock species. Seasonal differences of *E. coli* O157:H7 shedding in dairy and beef cattle have been well documented, with a greater prevalence of faecal shedding observed during the spring and summer months (Hancock *et al.* 1997; Barkocy-Gallagher *et al.* 2003; Miller *et al.* 2003), when ambient temperature and relative humidity are high and the day is longer. Two glands, which respond to photoperiod and demonstrate circannual

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rhythms in hormone synthesis and secretion include the thyroid (Souza *et al.* 2002) and pineal (Bubenik *et al.* 1998).

The thyroid gland and its associated hormones, triiodothyronine ($T_3$) and thyroxine ($T_4$), play a major role in controlling the body's metabolic activity (Griffen 1996). Additionally, the thyroid gland has been associated with seasonal changes in reproductive activity of the ram and ewe, specifically the transition from oestrus to anoestrus (Moenter *et al.* 1991; Karsch *et al.* 1995; Parkinson and Follett 1995). Seasonal variations in total serum $T_3$ and $T_4$ have been observed in sheep, with lower concentrations detected during the spring and early summer months (Webster *et al.* 1991; Karsch *et al.* 1995). Conversely, others have found serum concentrations of $T_3$ and $T_4$ to be greatest during the same months or when the day is the longest (Lincoln *et al.* 1980; Menegatos *et al.* 1993; Souza *et al.* 2002). In addition to photoperiod, environmental factors have been associated with thyroid activity. Valtorta *et al.* (1982) evaluated the effects of ambient temperature (35°C) on plasma thyroxine levels in sheep. High ambient temperature resulted in decreased plasma $T_4$ concentrations within the first 15 days of a 30-day treatment period. This would support the idea that thyroid hormone levels are lower during summer months when ambient temperature is high. However, similar to photoperiod, contradictory research has indicated serum concentrations of $T_3$ and $T_4$ are the highest during the warmer months of the year (Menegatos *et al.* 1993; Souza *et al.* 2002).

The similarity between seasonal shedding of *E. coli* O157:H7, and circannual rhythms of the thyroid hormones, led us to investigate the potential role of the thyroid in influencing seasonal shedding of *E. coli* O157:H7 in naturally infected cattle. The synthetic compound, 6-*N*-propyl-2-thiouracil (PTU), a potent thionamide has been utilized in human medicine and animal studies to suppress thyroid activity. In cattle, a dose of 4 mg PTU$kg^{-1}$ body weight (BW), administered for 35 days, resulted in altered thyroid function (De Moraes *et al.* 1998; Thrift *et al.* 1999a, 1992b). The objective of this experiment was to examine reduced thyroid activity and subsequent effects on faecal shedding patterns of *E. coli* O157:H7 and EC in cattle. We hypothesize that a higher PTU dose, administered for 14 days, would result in a gradual reduction in thyroid activity, as can be observed naturally in response to changing day length in turn decreasing the number of animals shedding *E. coli* O157:H7.

## MATERIALS AND METHODS

Eight yearling crossbred cattle (initial BW 295·3 ± 6·1 kg) were studied in this experiment. All procedures were preapproved by the Animal Care and Use Committee of the USDA–ARS, Southern Plains Agricultural Research Center. Cattle were assigned randomly to pens and pens assigned randomly to treatment groups ($n = 4$ per treatment group) with sex equally represented among treatment groups. All animals were housed in outdoor pens, received a finishing diet (80% concentrate, 20% grass hay) at 3·0% of BW, and had *ad libitum* access to water. Cattle were adapted to their pens and diets over a 14-day adjustment period. Treatments were control [CONT; 0 mg PTU; (Sigma Chemical Company, St Louis, MO, USA) $kg^{-1}$ BW $day^{-1}$] or PTU (10 mg PTU $kg^{-1}$ BW $day^{-1}$) administered daily for 14 days. Each animal's PTU dose was mixed with molasses and a small portion of the concentrate. This was fed to the cattle at 0800 h prior to receiving their full feed allotment to ensure each animal consumed their respective dose of PTU. Animals were sampled for faecal *E. coli* O157:H7 and *E. coli* during the 14-day treatment period, and an additional 7 days thereafter (post-treatment period). Body weights were recorded weekly. To determine serum concentrations of $T_3$ and $T_4$, blood was collected via jugular venipuncture on day −14, −7, 0, 3, 6, 9, 12, 15, 18 and 21 of the experiment. Blood was centrifuged at 3000 *g* and serum was stored at −20°C for hormone analysis. Serum $T_3$ and $T_4$ were determined by radioimmunoassay using components of commercial kits provided by Diagnostic Products Corporation (Los Angeles, CA, USA) which were validated for use in ruminant serum as described previously (Richards *et al.* 1999; Wells *et al.* 2003). Within and between assays, coefficients of variation for both methods were <5·0%. Faecal samples, acquired from the pen floor immediately after defecation or via rectal palpation, using a new shoulder-length glove, were obtained for quantification of *E. coli* O157:H7 daily and EC populations on day −7, 0, 7, 14 and 21. *Escherichia coli* O157:H7 was isolated using the technique described by Elder *et al.* (2000). Briefly, 10 g of faeces were enriched in 90 ml GN Hajna broth (Fisher Scientific, Hampton, NH, USA), containing cefixime (1·4 mg $ml^{-1}$), vancomycin (8·0 mg $ml^{-1}$) and cefsoludin (10 mg $ml^{-1}$) (Sigma Chemical Company), and incubated for 6 h at 37°C. One-millilitre aliquots of enriched broth were concentrated using immunomagnetic beads (Neogen Corp., Lansing, MI, USA) specific for the O157 antigen. Immunomagnetic separation beads were plated on CHROMagar O157 (CHROMagar Microbiology, Paris, France) agar and incubated (24 h, 37°C). Suspect colonies were picked and verified as *E. coli* O157:H7 using Reveal$^{TM}$ *E. coli* O157:H7 test strips (Neogen).

For quantification of EC, 1 g faeces was serially diluted in phosphate-buffered saline (PBS), plated on CHROMagar *E. coli* (CHROMagar Microbiology) agar and incubated at 37°C for 24 h.

Body weights, serum $T_3$ and $T_4$ were analysed using the MIXED procedure of SAS (SAS Institute Inc, Cary, NC, USA) with repeated measures. Treatment, day and treat-

ment × day interaction were included in the model. Daily faecal shedding of *E. coli* O157:H7 was analysed using frequency analysis and the GENMOD procedure of SAS with repeated measures. Treatment and day were included in the model. A *P*-value <0·05 was declared significant.

## RESULTS

Faecal shedding of *E. coli* O157:H7 varied daily throughout the experimental period within treatment groups (Fig. 1). When averaged over the 14-day treatment period, shedding of *E. coli* O157:H7 was similar ($P = 0{\cdot}31$) between treatments with 54·6 and 40·4% of PTU- and CONT-treated cattle shedding respectively. However, during the post-treatment period, a higher percentage of PTU cattle tended to shed *E. coli* O157:H7 on day 16 (100 *vs* 25%; $P = 0{\cdot}07$) and 18 (75·0 *vs* 0%; $P = 0{\cdot}07$) compared with CONT animals.

Treatment had no effect ($P = 0{\cdot}76$) on shedding of EC. Populations on day 0, prior to PTU treatment, averaged 6·4 and 6·0 CFU $g^{-1}$ faeces ($log_{10}$) for CONT and PTU animals respectively. *Escherichia coli* on day 14 [6·9 and 6·3 CFU $g^{-1}$ faeces ($log_{10}$) and 21 (6·3 and 6·6 CFU $g^{-1}$ faeces ($log_{10}$)] were not different ($P > 0{\cdot}10$) between treatments after exposure to 0 or 10 mg PTU $kg^{-1}$ BW $day^{-1}$ for CONT- and PTU-treated cattle respectively.

A treatment × day interaction ($P \leq 0{\cdot}01$) was detected for both serum $T_3$ and $T_4$ concentrations (Fig. 2). Concentrations of $T_3$ in PTU-treated cattle were lower over much of the 14-day treatment period compared with CONT animals. PTU-treated cattle had lower levels of $T_3$ on day 6 (1·35 *vs* 1·68 ng $ml^{-1}$; $P = 0{\cdot}03$), 9 (1·02 *vs* 1·33 ng $ml^{-1}$; $P = 0{\cdot}04$), and 12 (0·95 *vs* 1·28 ng $ml^{-1}$; $P = 0{\cdot}03$). Triiodothyronine concentrations were similar ($P = 0{\cdot}3$) between treatment groups on day 15 and 21 postPTU treatment, but were

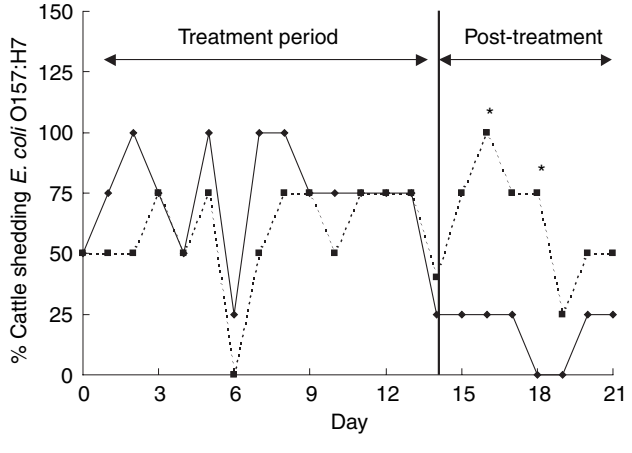


**Fig. 1** Faecal shedding of *Escherichia coli* O157:H7 for cattle receiving 0 (——) or 10 (- - - -) mg 6-*N*-propyl-2-thiouracil (PTU) $kg^{-1}$ BW $day^{-1}$ for 14 days. *$P < 0{\cdot}05$

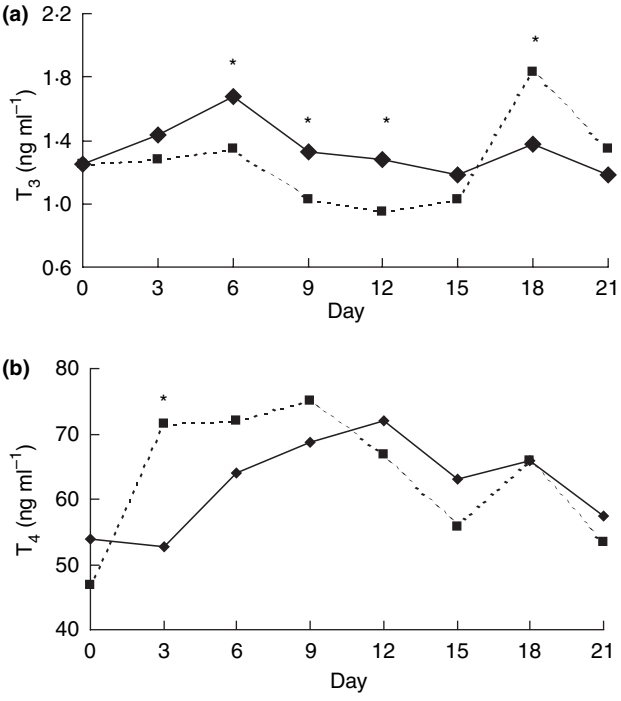


**Fig. 2** Total serum triiodothyronine ($T_3$; a) and thyroxine ($T_4$; b) for cattle receiving 0 (——) or 10 (- - - -) mg 6-*N*-propyl-2-thiouracil (PTU) $kg^{-1}$ BW $day^{-1}$ for 14 days. *$P < 0{\cdot}05$

higher ($P = 0{\cdot}004$) for PTU-treated cattle (1·83 *vs* 1·38 ng $ml^{-1}$) on day 18 (3 days after cessation of PTU treatments (Fig. 2a). In PTU-treated cattle, serum concentrations of $T_4$ increased within the first 3 days of receiving their treatment and differed from CONT animals on day 3 (71·5 *vs* 52·8 ng $ml^{-1}$; $P = 0{\cdot}02$); however for the remainder of the sampling period, concentrations were similar between treatment groups.

Body weights were similar ($P = 0{\cdot}48$) between treatment groups on day 0 (306·6 *vs* 284·7 kg for CONT and PTU respectively). Although animals within both treatment groups gained weight during the 14-day treatment period, as well as lost weight during the post-treatment period, no differences in BW gain were observed between treatments for either period.

## DISCUSSION

Variable shedding patterns of *E. coli* O157:H7 has been documented in both young and adult cattle. Robinson *et al.* (2004) sampled calves up to five times per day for 5 days, and once or twice a day for 15 days, and found the prevalence of shedding on any one visit varied between 20 and 90% with no obvious diurnal trend. Some animals were even sampled twice during a single visit and exhibited marked variability in the concentration of *E. coli* O157:H7

shed on that single day. In the current study, regardless of their treatment, animals typically shed *E. coli* O157:H7 50% of the time over the 21-day sampling period with intermittent shedding exhibited by all animals.

In addition to circadian shedding patterns, seasonal shedding of *E. coli* O157:H7 is well documented in cattle with the greatest prevalence typically observed during the spring and summer months (Hancock *et al.* 1997; Barkocy-Gallagher *et al.* 2003; Miller *et al.* 2003), when ambient temperature, relative humidity, and day length are increased. A 14-herd study (Hancock *et al.* 1997) evaluating the prevalence of *E. coli* O157 in naturally infected cattle, found animals sampled on a monthly basis for 2 years were intermittent shedders with the greatest prevalence observed during the summer months (2·6% in June) and the lowest in December. The authors suggest the typical pattern of *E. coli* O157 shedding by cattle appears to be characterized by short periods with a relatively high prevalence separated by longer periods of diminished or undetectable shedding.

The thyroid is one of several glands that respond to day length and ambient temperature although previous research is contradictory. Lincoln *et al.* (1980) and Souza *et al.* (2002) reported serum concentrations of $T_3$ and $T_4$ were the greatest during the spring and early summer months, which parallels the increase in faecal shedding of *E. coli* O157:H7 exhibited in cattle (Barkocy-Gallagher *et al.* 2003; Miller *et al.* 2003). On the contrary, Webster *et al.* (1991) and Karsch *et al.* (1995) observed decreased serum concentrations of $T_3$ and $T_4$ during the warmer months of the year. The goal of the current study was to induce a mild depression in thyroid activity as might occur naturally because of changing day length. This was demonstrated by decreased serum concentrations of $T_3$ observed during the PTU treatment period. However, no differences in faecal shedding of *E. coli* O157:H7 were observed. Interestingly, when PTU treatments were terminated, serum concentrations of $T_3$ increased above controls and differed by day 18. Also during this time, shedding tended to be greater on day 14 and 16 for PTU-treated cattle whereas faecal shedding of *E. coli* O157:H7 decreased in CONT cattle. The slight differences in shedding patterns observed between groups during the post-treatment period may be a result of the inherent variation associated with daily shedding patterns. Although PTU-treated cattle maintained a level of shedding during the post-treatment period similar to that in the treatment period, the simultaneous increase in serum $T_3$, and tendency for higher shedding of *E. coli* O157:H7 by PTU-treated cattle from day 14 to 16 *vs* that observed by CONT cattle, warrants further research to determine if the thyroid is involved with seasonal shedding of *E. coli* O157:H7.

Differences in ambient temperature, and to some extent day length, were observed during the study. Ambient temperature during the treatment and post-treatment periods differed with the highest average maximum temperature (33·6°C) occurring while animals received their respective treatments. Temperatures decreased during the post-treatment period with an average maximum temperature of 31·5°C. This decrease in temperature and concurrent increase in $T_3$ would agree with Webster *et al.* (1991) who propose plasma concentrations of $T_3$ vary inversely with ambient temperature, suggesting temperature is associated with the increase in serum $T_3$ observed in the post-treatment period. However, temporary hyperthyroidism is a common side effect in humans who have PTU treatments terminated (LaFranchi and Mandel 1996) and has also been commonly observed in animal studies utilizing PTU (Thrift *et al.* 1999a, 1999b; Bollinger *et al.* 2000). Thus, the spike in serum levels of $T_3$ and $T_4$ observed in the post-treatment period is independent of ambient temperature.

Faecal EC populations were within ranges reported for cattle consuming a high concentrate diet (Diez-Gonzalez *et al.* 1998; Stanton and Schutz 2000) with total EC populations averaging 6·9 log cells $g^{-1}$ faeces for cattle in this experiment. Diez-Gonzalez *et al.* (1998) reported total *E. coli* counts of 6·8 and 6·9 log cells $g^{-1}$ faeces for cattle consuming a moderate grain or high grain diet, respectively, thus suggesting, no direct effects of PTU on EC shedding.

Increased gains in BW have been observed with cattle receiving PTU (Bernal *et al.* 1999; Thrift *et al.* 1999a, 1999b). However, animals in this experiment were administered PTU for a period that was only half of that reported in the literature evaluating the effects of hypothyroidism on reproductive performance of heifers immediately following calving (Bernal *et al.* 1999; Thrift *et al.* 1999a, 1999b). Although PTU-treated cattle gained an average of 9·0 kg, this was not a significant difference.

The utilization of PTU to reduce thyroid activity did not appear to directly affect shedding patterns of *E. coli* O157:H7 or commensal *E. coli* in yearling cattle consuming a high-concentrate diet. However, a change in *E. coli* O157:H7 shedding and an increase in $T_3$ concentrations were observed following cessation of PTU treatments. Animals naturally infected with *E. coli* O157:H7 were utilized which limited the number of animals available for this study. This may have limited our sensitivity and possibly the ability to detect small differences. Furthermore, we attempted to accomplish in a couple of weeks (altered thyroid function) what occurs naturally over the course of several months. Increasing the duration of PTU may have resulted in a greater separation of serum $T_3$ and $T_4$ concentrations between treatment groups, and consequently, resulted in greater differences in faecal shedding of *E. coli* O157:H7 during the PTU treatment period. However, despite the limitations, we feel the methods used herein provided a realistic approach to examining the role of the thyroid on *E. coli* O157:H7. To determine if

differences in faecal shedding of *E. coli* O157:H7 is related to thyroid function or intermittent shedding better, further research is needed.

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